REMARKS

Reconsideration and withdrawal of the rejections set forth in the Office action dated November 19, 2002 are respectfully requested.

Attached is a separate page captioned **"Version with Markings to Show Changes Made** with a marked-up version of the claims to show the changes made.

I. Amendments

A. In the Specification:

The specification has been amended to clarify that the bars of Figures 13A, 13B, 14, 15, and 18A-18C represent different sample runs at the same concentration.

The specification has further been amended to correct an obvious typographical error.

B. In the Claims:

Claims 1, 13-16, and 18-19 are amended to clarify that the gene transfer vector comprises an exogenous gene encapsulated in a virus envelope membrane. Support for this amendment can be found on page 15, lines 11-14.

No new subject matter has been added by way of these amendments.

II. Priority

Applicant encloses herewith a certified copy of the translation of the foreign priority document JP 2000-25596 filed 2/2/2000. Accordingly, Applicant submits the application complies with all the requirements for receiving the benefit of priority under 35 USC §119.

III. Objections

The Examiner objected to the disclosure for alleged informalities.

The Examiner objected to Figure 7 for allegedly not having a distinct band observed. Applicant does not understand this rejection. Applicant sees distinct bands at each of the proteins HN and F1 described in the specification, as indicated by arrows in

Figure 7. If the Examiner would prefer the Figure as a photograph or an artist rendering, either can be provided.

The Examiner objected to Figures 13, 15, and 18 for allegedly not having a label for the different bars. The Examiner further objected to Figure 14 as allegedly unclear what bar-1 and bar-2 stand for. The bars in Figures 13A, 13B, 14, 15, 18A and 18B represent multiple sample runs at the same concentration. The specification has been amended to clarify this fact.

To the extent objections to the figures remain after consideration of the preceeding amendments and comments, Applicant respectfully requests any such objections be held in abeyance until such time as there is allowable subject matter.

The Examiner objected to the description of Figure 14 as allegedly unclear whether the data represents *in vivo* or *in vitro* experiments. The specification has been amended to clarify that the squamous cell carcinoma is from a human tongue. The description of the Figure has additionally been amended to clarify that the gene transfer is performed *in vitro*.

The Examiner objected to the description on page 41, lines 5-9 as allegedly unclear whether the method is for *in vivo* or *in vitro*. As noted above, the specification has been amended to clarify that this example is conducted *in vitro*.

IV. Rejections under 35 U.S.C. §102

Claims 1-14 and 20-29 were rejected under 35 U.S.C. §102(e) as allegedly anticipated by Hoon et al. (U.S. Patent No. 6,472,375).

Claims 1-14, 18, 20-29 and 31-33 were rejected under 35 U.S.C. §102(e) as allegedly anticipated by Olson (U.S. Patent No. 6,372,957).

These rejections are respectfully traversed for the following reasons.

A. The Invention

The present invention relates to a gene transfer vector comprising an exogenous gene encapsulated in a virus envelope membrane (claim 1), a pharmaceutical composition (claim 13) and a kit (claim 14) utilizing the vector as well as a method for

preparing the gene transfer vector (claims 15-16), and a method for introducing an exogenous gene into a suspended cell using the gene transfer vector (claim 19).

B. The Cited Art

HOON ET AL. relates to a viral liposome composition comprising a liposome fused to an inactivated virus and comprising a nucleic acid, preferably DNA encoding a tumor-associated antigen.

OLSON relates to methods of treating cardiac hypertrophy as well as transgenic constructs for preparing transgenic animals. The transgenic animals or cells from the animals are used for the detection of compounds having therapeutic activity toward cardiac hypertrophy. It is disclosed that the transgenic expression construct may be entrapped in a liposome. The liposome may further be complexed with a hemagglutinating virus.

C. Analysis

1. Legal Standard

The standard for lack of novelty, that is, for anticipation, is one of strict identity. To anticipate a claim for a patent, a single prior source must contain all its essential elements. M.P.E.P. § 2131.

2. Rejection over Hoon et al.

Hoon et al. fail to teach a gene transfer vector comprising an exogenous gene encapsulated in a virus envelope membrane. Hoon et al. teach a viral liposome composition comprising a liposome fused to an inactivated virus and comprising a nucleic acid, preferably DNA encoding a tumor-associated antigen.

3. Rejection over Olson

Olson fails to teach a gene transfer vector comprising an exogenous gene encapsulated in a virus envelope membrane. The expression construct of Olson may

be entrapped in a liposome, which may further be complexed with a hemagglutinating virus.

As the standard for novelty has not been satisfied, withdrawal of the rejections under 35 U.S.C. §102 is respectfully requested.

V. Rejections under 35 U.S.C. §103

Claims 19 and 34-36 were rejected under 35 U.S.C. §103 as allegedly obvious over Hoon et al. in view of Jessee (U.S. Patent No. 6,020,202) and further in view of Dubensky, Jr. *et al.* (U.S. Patent No. 6,451,592).

Claims 15-17 and 30 were rejected under 35 U.S.C. §103 as allegedly obvious over Jessee in view of Fields *et al.* (Virology, Lippincott Williams & Wilkins, 1996, p. 1178) and Harmsen *et al.* (*Eur J. Biochem*, 149:591-600, 1985).

These rejections are respectfully traversed for the following reasons.

A. The Invention

The present invention is described above.

B. The Cited Art

HOON ET AL. are described above.

OLSON is described above.

JESSEE relates to a composition for transfection of eukaryotic cells. The composition comprises a cationic lipid aggregate complexed with nucleic acids. The aggregate complex further includes an enveloped virus for enhancement of transfection.

DUBENSKY, JR. ET AL. describe a vector comprising an isolated nucleic acid molecule comprising an alphavirus nonstructural protein that is incorporated into a particle. The vector can be used as a pharmaceutical composition that may be provided

as a liposomal formulation. The vector may be complexed with a commercially-available lipid such as lipofectin or lipofectamine for transfection.

FIELDS ET AL. relates to Paramyxoviridae viruses and their replication. Particularly noted by the Examiner is that Paramyxoviridae contain a lipid bilayer envelope that is derived from the plasma membrane of the host cell.

HARMSEN ET AL. describe reconstitution of a Sendai virus membrane using detergent dialysis.

C. Analysis

1. Rejection over Hoon et al. in view of Jessee and further in view of Dubensky, Jr. *et al.*

According to M.P.E.P. §2143, one of the three requirements to establish a case of *prima facie* obviousness, is that the prior art references teach or suggest all the limitations of the claim.

As detailed above, Hoon et al. fail to show or suggest a gene transfer vector comprising an exogenous gene encapsulated in a virus envelope membrane.

The cited Jessee and Dubensky, Jr. et al. references, alone or in combination, do not make up for this deficiency. Jessee is concerned with a cationic lipid aggregate complexed with nucleic acids, where the complex may further include an enveloped virus. Dubensky, Jr. et al. is concerned with a vector that may be included in a pharmaceutical composition that may be provided as a liposomal formulation or that the vector may be complexed with a commercially available lipid.

Further, the Examiner states that it would have been prima facie obvious to use the vector taught by Hoon et al. to transfect suspension cells as taught by Jessee in the presence of protamine sulfate as taught by Dubensky, Jr. et al "since the vector taught by Hoon et al. contains reconstituted viral envelope." Applicant respectfully disagrees. Hoon et al. teach fusion of the HVJ reagents with the liposome. HVJ is inactivated by detergent, heat and UV light. The inactivated HVJ is added to a DNA:liposome solution and

incubated to create the fused HVJ-liposome. Free HVJ is removed from the solution. Nowhere does Hoon et al. teach reconstitution of the virus or the viral envelope.

2. Rejection over Jessee in view of Fields *et al.* and Harmsen *et al.*

a. The Cited Documents Fail to Teach or Suggest all the Claim Limitations

As noted above, according to M.P.E.P. §2143, one of the three requirements to establish a case of *prima facie* obviousness, is that the prior art references teach or suggest all the limitations of the claim.

The invention recites a method for preparing "a gene transfer vector comprising an exogenous gene encapsulated in a virus envelope membrane". The method, according to claim 15, comprises mixing the virus with an exogenous gene and freezing and thawing the mixture two or more times. The method, according to claim 16, comprises mixing the virus with an exogenous gene in the presence of a detergent.

Nowhere is preparation of a gene transfer vector comprising an exogenous gene encapsulated in a virus envelope membrane shown in the cited documents, when considered alone or in combination. Jessee teach preparation of a cationic lipid aggregate complexed with nucleic acids, where the complex may further include an enveloped virus, not an exogenous gene encapsulated in a virus envelope membrane.

Neither Fields et al. nor Harmsen et al., alone or in combination, make up for this deficiency. Fields et al. is a treatise on Paramyxoviridae viruses and their replication. The Examiner cites the reference for a teaching that the viruses contain a lipid bilayer envelope that is derived from the plasma membrane of the host cell. Harmsen et al. describe reconstitution of a Sendai virus membrane using detergent dialysis. Neither reference teach a gene transfer vector comprising an exogenous gene encapsulated in a virus envelope membrane.

b. There is no Motivation to Modify or Combine the References

According to M.P.E.P. §2143, to establish a case of *prima facie* obviousness there must be some suggestion or motivation, either in the references themselves or in

the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings.

Even if the combination of Jessee in view of Fields *et al.* and Harmsen *et al.* taught or suggested all limitations of the claimed invention, none of the references, or combination of references, provides motivation for combining the references along the lines of the invention.

To arrive at the claimed invention, one would need to modify Jessee in two ways. First, it would be necessary to exchange the lipid aggregate, which includes liposomes, of Jessee for a virus envelope membrane. Second, one would need to modify Jessee to encapsulate an exogenous gene in the virus envelope membrane.

Motivation to make each of these modifications is simply not found in Jessee, Fields et al., or Harmsen *et al.* Jessee teaches transfection using a cationic lipid complex. The complex may further include a virus envelope or component thereof within the complex to enhance transfection. Jessee further provides no guidance for encapsulating an exogeneous gene in a virus envelope membrane. In fact, Jessee teaches that "[u]nlike prior art methods, the enhanced transfection methods of this invention do not require encapsulation of the nucleic acid within anionic phospholipid-based liposomes" (Col. 3, lines 63-66). Jessee makes no mention of using a virus envelope membrane as anything other than an addition to the lipid complex to enhance transfection.

Nor is motivation for the modifications found in Fields *et al.* or Harmsen et al. Fields et al. is cited for a teaching that the viruses contain a lipid bilayer envelope that is derived from the plasma membrane of the host cell. A mere teaching that a virus envelope is a lipid bilayer does not teach equivalence to a liposome or a cationic liposome capable of complexing with a nucleic acid, much less that the lipid bilayer could be used to encapsulate an exogenous gene. In fact, Jessee teaches "[c]ationic lipids are not universally effective for transfection of all cell types. Effectiveness of transfection of different cells depends on the cationic lipid composition used and the type of lipid aggregate formed" (Col. 1, lines 50-53). Harmsen et al. merely describe reconstitution of a Sendai virus membrane using detergent dialysis. No mention is

made of encapsulation, transfection, or of using a virus membrane for either encapsulation or transfection.

The Examiner states that "one of skill in the art of gene transfer would be motivated to combine the teachings of Jessee and Fields et al. to omit lipid used in the vector preparation by mixing envelope virus with exogenous nucleic acid directly"..."to incorporate the nucleic acid with virus lipid bilayer envelope and to inactivate the virus at the same step." This is simply not true. Encapsulation of nucleic acids in a lipid bilayer is very difficult due to the size and random configuration of nucleic acids.

Accordingly, nothing in the teachings of Jessee *et al.*, Fields et al., or Harmsen et al. would motivate one skilled in the art to modify the teachings of Jessee *et al.* to encapsulate an exogenous gene in a virus envelope membrane.

In view of the above, Applicant submits that the standard for obviousness has not been met. Accordingly, Applicant respectfully requests withdrawal of the rejections under 35 U.S.C. §103.

VI. Conclusion

In view of the foregoing, Applicant submits that the claims pending in the application are in condition for allowance. A Notice of Allowance is therefore respectfully requested.

If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 838-4410.

Respectfully submitted,

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Date: 2/19/03

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

On page 13, please replace the paragraph starting on line 15 with the following:

Figures **18A-18C** show[s] the results of an introduction experiment on CCRF-CEM (Figure **18B**), NALM-6 (Figure **18A**), and K-562 (Figure **18C**), which are human leukemia cell lines.

On page 41, please replace the paragraph starting on line 4 with the following:

The results are shown in Figures **13A** and **13B**. The samples were run in triplicate and each bar at each irradiation concentration corresponds to the sample tested in triplicate.

On page 41, please replace the paragraph starting on line 8 with the following:

To a squamous cell carcinoma (SAS) [on]from a human tongue, gene transfer was performed *in vitro* according to the method described in Example 11. The results are shown in Figure **14**. The samples were run in duplicate and each bar at each protamine sulfate concentration and incubation time corresponds to the sample tested in duplicate. Upon gene transfer, the protamine sulfate concentration and the incubation time for transfection were varied as shown in Figure **14**, and the gene transfer efficiency was measured based on the expression of the luciferase gene. Under the conditions used for the transfection, the gene transfer efficiency was maximum in the case where a transfection treatment was carried out for 60 minutes by using 200 mg/ml of protamine sulfate. However, further increases in the gene transfer efficiency are expected by further increasing the protamine sulfate concentration.

On page 41, please replace the paragraph starting on line 23 with the following:

Gene introduction was performed for human aortic endothelial cells (HAEC) according to the method described in Example 11. The results are shown in Figure **15**. The samples were run in duplicate and each bar at each protamine sulfate concentration and incubation time corresponds to the sample tested in duplicate.

On page 48, please replace the paragraph starting on line 6 with the following:

The cell lines used (in particular CCRF-CEM and NALM-6) show a very low introduction efficiency in the case where HVJ-liposomes or existing liposome reagents (Lipofectamine, Lipofectin of Gibco BRL, etc.) are used. However, as shown in Figures **18A** and **18B**, a highly efficient gene transfer to these cell lines was observed. The samples were run in duplicate and each bar at each protamine sulfate concentration and centrifugation rpm corresponds to the sample tested in duplicate.

In the Claims:

1. (Amended) A gene transfer vector [containing] comprising an exogenous gene encapsulated in a virus envelope membrane.

13. (Twice Amended) A pharmaceutical composition for gene therapy which comprises a gene transfer vector [containing] comprising an exogenous gene encapsulated in a virus envelope membrane.

14. (Twice Amended) A kit for screening gene libraries, which comprises a gene transfer vector [containing] comprising an exogenous gene encapsulated in a virus envelope membrane.

15. (Amended) A method for preparing a gene transfer vector comprising an exogenous gene encapsulated in a virus envelope membrane for gene transfer, wherein the method comprises the steps of:

mixing the virus with [an]the exogenous gene; and

freezing and thawing the mixture two or more times.

16. (Twice Amended) A method for preparing a gene transfer vector comprising an exogenous gene encapsulated in a virus envelope membrane for gene transfer, wherein the method comprises the step of:

mixing the virus with [an]the exogenous gene in the presence of a detergent.

18. (Twice Amended) A method for introducing a gene into isolated animal tissue, wherein the method comprises the steps of:

preparing a gene transfer vector [containing] comprising an exogenous gene encapsulated in a virus envelope membrane [and a desired exogenous gene]; and

introducing the [a] exogenous gene into the isolated animal tissue via the gene transfer vector.

19. (Twice Amended) A method for introducing an exogenous gene into a suspended cell, wherein the method comprises the steps of:

mixing the suspended cell with a gene transfer vector [containing] comprising the exogenous gene encapsulated in a virus envelope membrane in the presence of protamine sulfate; and

centrifuging the mixture.